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Rats heterozygous for the MENX-associated p27 mutation develop a MEN phenotype

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Background

We recently identified a variant of the MEN syndromes that spontaneously developed in a rat colony. This rat syndrome was first reported to be inherited in a recessive fashion. Affected animals (homozygous mutant) show phenotypic overlap with both the MEN1 and MEN2 human syndromes in that they develop tumors in adrenal glands, pituitary, thyroid, parathyroid and endocrine pancreas (see **Fig.1**). Due to the unique combination of affected organs of this multi-tumor syndrome, we named it MENX.

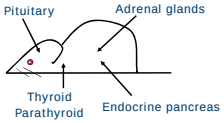

Fig.1 *– Schematic representation of endocrine tumor pattern in MENX rats.*

The MENX syndrome is caused by an inactivating germline mutation of the *Cdkn1b* gene, encoding the cell cycle inhibitor p27: affected rats are homozygous for a tandem duplication of 8 nucleotides in exon 2 of *Cdkn1b*. Immunohistochemical staining using an anti-p27 specific antibody showed that there is lack or extreme reduction of the p27 protein in tissues of affected rats compared to the expression pattern of the normal protein in unaffected rat tissues (see **Fig.2**).

Interestingly, we could further demonstrate that *CDKN1B* is a tumor susceptibility gene also in humans as germline alterations have been identified in patients having MEN1-like features but no *MEN1* mutations. This newly identified syndrome was named multiple endocrine neoplasia type 4 (MEN4).

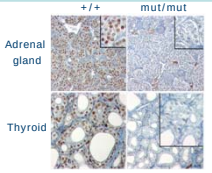

Fig.2 *– Anti-p27 IHC in tissues of MENX rats.*

Aims

As many data coming from both animal models and MEN4 patients tend to attribute an important role of a single absent/mutated p27 allele in neuroendocrine tumorigenesis, we decided to perform a detailed analysis of the phenotype of rats heterozygous for the germline mutation in *Cdkn1b* causing the MENX syndrome.

Results

Two cohorts of rats either heterozygous for the *Cdkn1b* MENX-related germline mutation (p27+/mut) or wild-type (p27+/+) were generated and animals were kept alive until they showed signs of distress. The cumulative survival curves (see **Fig.3**) clearly showed that the heterozygous rats live significantly shorter than their wild-type littermates (p=5,62 e-08).

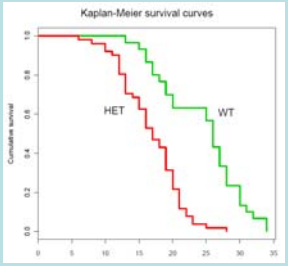

Fig.3 *– Kaplan-Meier survival curves (months) for p27+/mut and p27 +/+ rats.*

To understand why the heterozygous rats were dying earlier than the wild-type animals, we performed complete necropsy and histological examination of the tissues.

We observed that, differently from what was till now described, p27+/mut rats developed adrenomedullary tumors, pituitary tumors, medullary thyroid tumors and parathyroid hyperplasia, like the double mutants: p27+/mut rats were significantly more susceptible than wild-type rats to tumorigenesis (see **Fig.4a**).

This observation is particularly evident if one looks at the data regarding pituitary and thyroid tumors (see **Fig.4b**): the animals are younger at disease onset and the tumor multiplicity is higher.

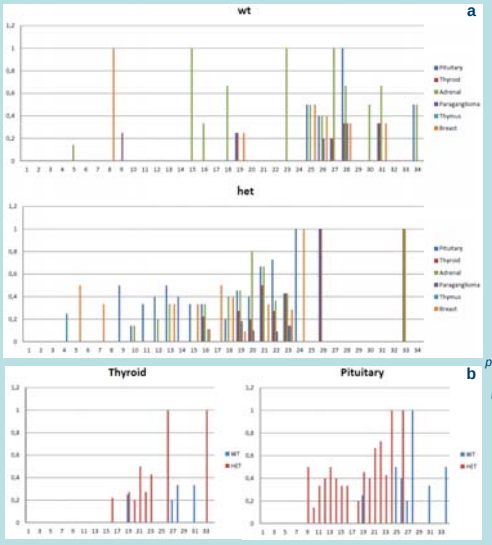

Fig.4
a. *Presence of macroscopic tumors at necropsy in p27+/mut and p27 +/+ rats;*
b. *details for lesions in pituitary gland and thyroid.*

As already observed for the double mutants, also in the heterozygous rats the hyperplasias/neoplasias affecting adrenal, parathyroid, and medullary thyroid tissues were almost exclusively bilateral.

Since the lifespan of the heterozygous rats is longer than that of the double mutants, some of the tumors they developed reached a considerable size.

For many tumor types it was possible to observe the various steps of tumorigenic progression. In particular, we could observe all the phases of progression of medullary thyroid carcinoma (MTC), from C-cell hyperplasia to carcinoma, often within the same gland (see **Fig.5**).

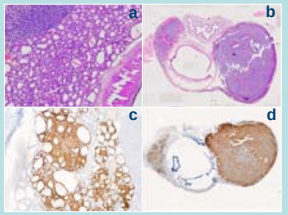

Fig.5 *– **a-b.** H&E staining of thyroid tissues presenting C-cell hyperplasia (**a**) and adenoma/carcinoma (**b**); **c-d.** anti-calcitonin immunostaining of thyroid tissues presenting C-cell hyperplasia (**c**) and carcinoma (**d**).*

Conclusions and future perspectives

Rats heterozygous for the germline mutation in *Cdkn1b*, causing the MENX syndrome, develop a MEN phenotype, like the double mutant animals.

Since the MENX rat model system offers the unique opportunity to dissect at the molecular level the different stages of neuroendocrine cell transformation, we would like to exploit this model system to gain information about neuroendocrine tumorigenesis in both rodents and humans. In particular, we want to improve our understanding of medullary thyroid carcinoma development and progression through miRNA and mRNA expression profiling.

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